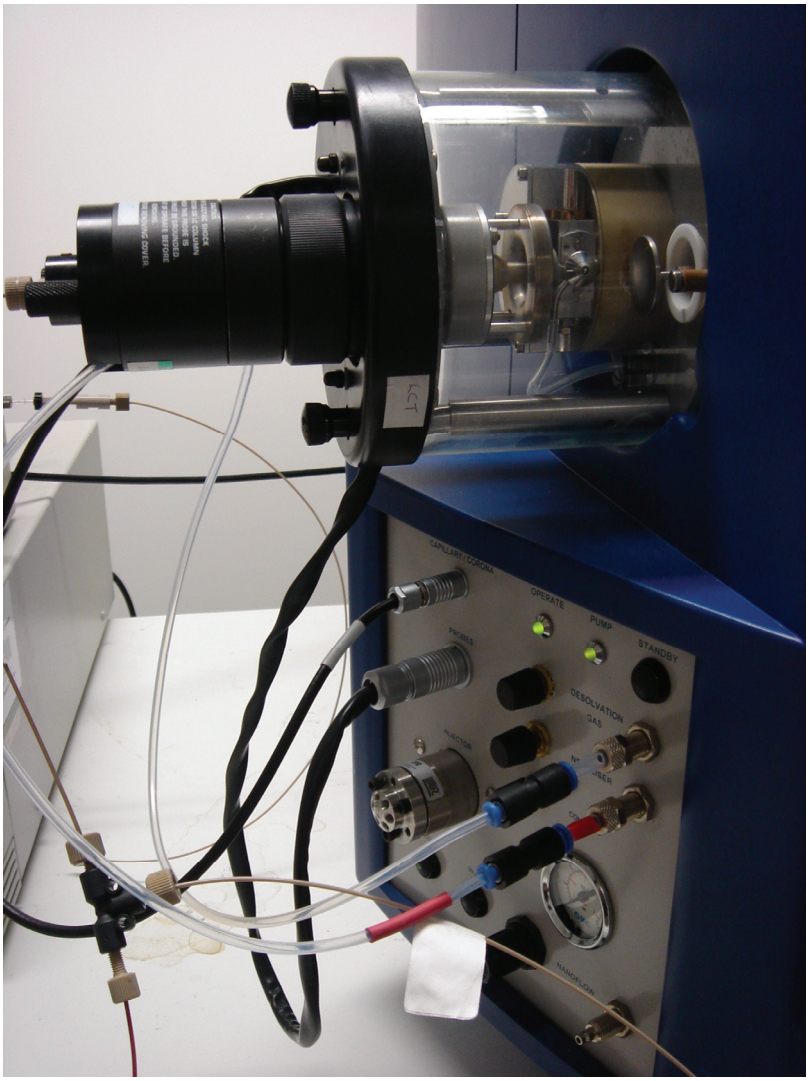
# CRL MASS SPECTROMETRY FACILITY
# USER MANUAL

# LCT CLASSIC A & B




## 1st Floor Mass Spec Lab: 00.097

This is a guide to using the LCT classic for those who have received training. If you have any questions or problems whilst using this instrument please contact a member of the CRL Mass Spectrometry Facility Staff.

CONTENTS



## 1. Check the system is working properly

*Make sure the Masslynx program is running and in operate with the appropriate .IPR file (masslynx method) loaded for positive or negative ion mode. (Note when you have finished using the instrument it should be left with the masslynx program running and in operate.*

1.1 Open your masslynx project: File/Open project/select your project name (Fig 1). A warning message will appear, click 'yes'.

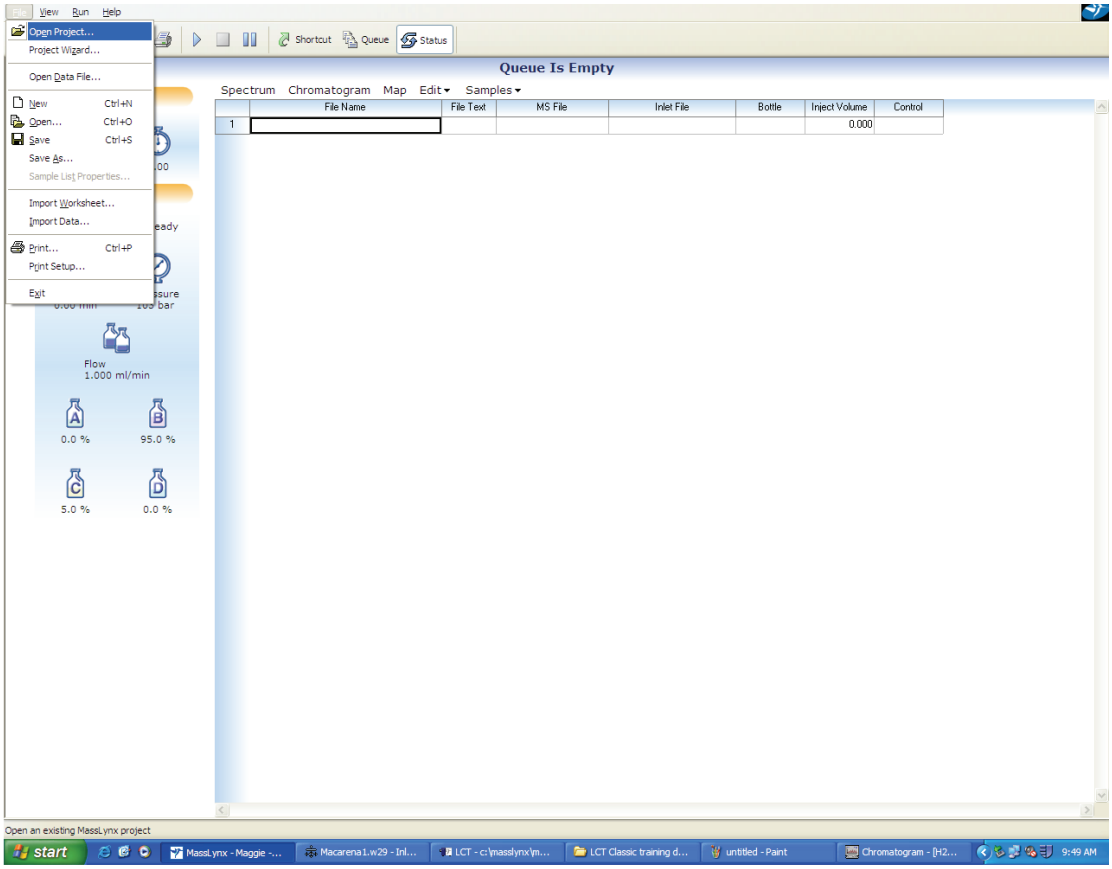



**Figure 1: Open your masslynx project.**

1.2 Is the sample cone clean (B in figure 2)? Any visible browning or other discolouration/deposit close to the cone's orifice will reduce the sensitivity of

the instrument. It is your responsibility to ensure the instrument, and the cone, is left clean such that it will not impair sensitivity for the next user (Fig 2).

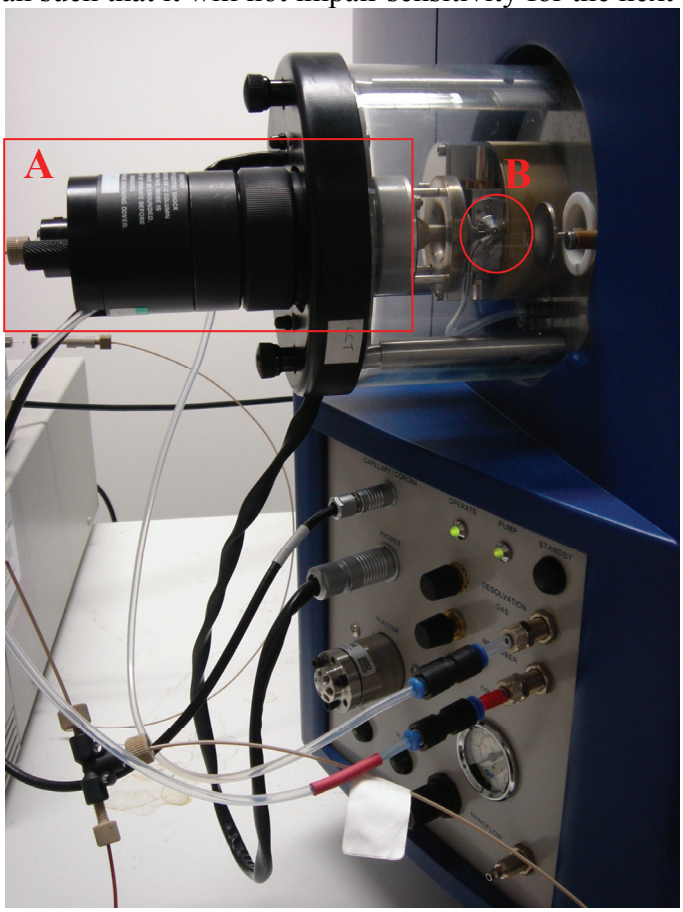



**Figure 2: ESI probe (A) and cone (B).**

1.3 Choose and load a suitable IPR file for your experiment then select the corresponding ion mode (Fig 3 & 4).

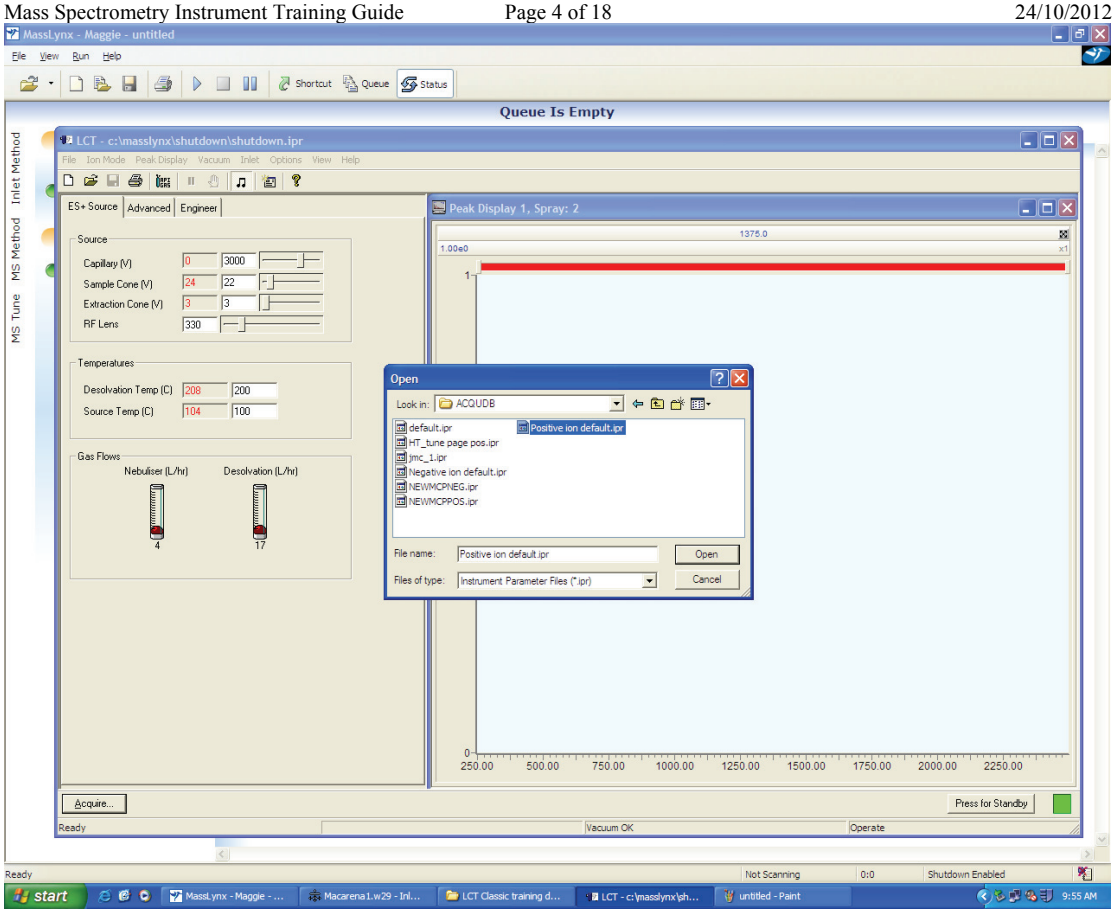



**Figure 3: Select a suitable IPR ('tuning') file for your experiment from File/open (make sure your project is selected).**

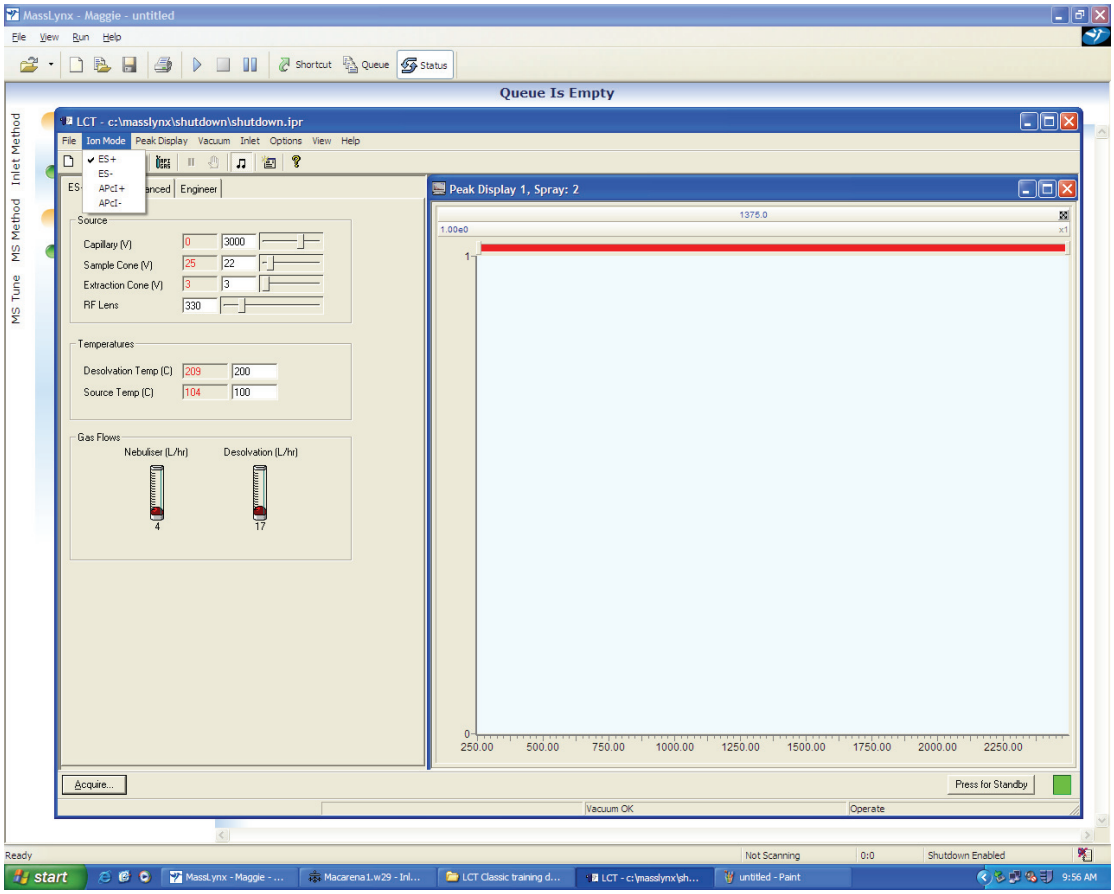



**Figure 4: Choose the correct ion mode for your experiments (Ion mode ES+ or ES-)**

1.4 Before setting up a sequence of injections you should directly infuse a standard sample to check the mass spec is functioning normally and to calibrate if necessary. For small molecule LC/MS it is suggested to use sodium formate at a concentration of 15ug/mL in 50:50 can/Water with 0.1% formic acid (this covers a mass range from m/z 91-m/z 2062 however it is more difficult to get sufficient ions to calibrate masses above m/z 1500).

## 2. Calibrating the instrument with Myoglobin or Sodium formate

2.1 To calibrate for denatured protein analysis another protein with a charge state envelope covering the expected mass range can be used. BSA and myoglobin are common examples. Myoglobin will provide a charge state from about m/z 700-2000 (Figure 7).

2.2 If it is not already in operate switch into operate and switch on API (A & B; Figure 5).

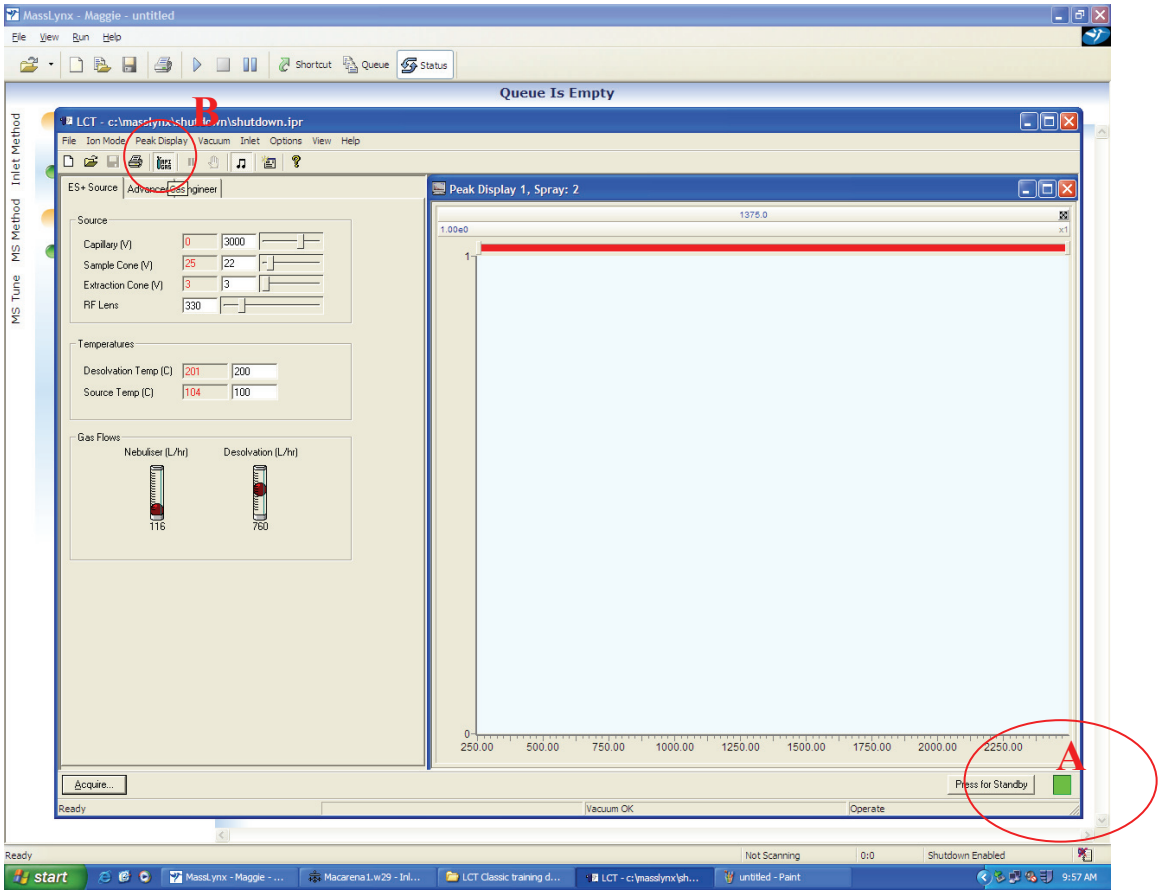



**Figure 5: Switch into operate (A), check gas on (B)**

2.3 Directly infuse the appropriate standard solution at about 0.01-0.1mg/mL or ~10uM using the syringe pump and a 250uL syringe.

2.4 Acquire data manually for directly infused sample (Figure 6).

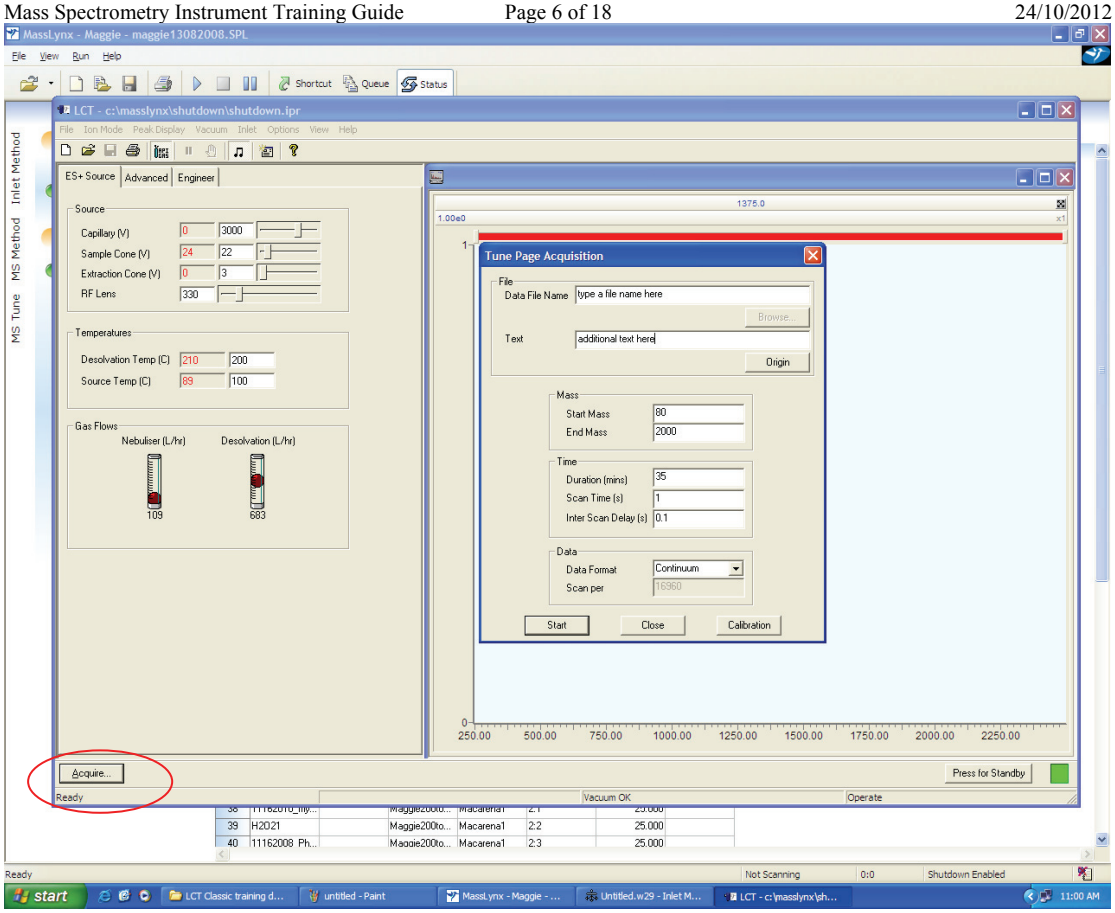



**Figure 6: Acquire data manually**

2.5 In order to check the mass for Myoglobin de-convolute the charge state envelope: First process (smooth, subtract and centre using the 'process' tab. Then select process/components and manual (fill data in). Alternatively the Maxent 1 tool can be used to de-convolute the spectra. (Note: this works by taking the spectra actually displayed in the mass spectrum window and not all the date saved in the data file). Relevant reference values for the ions in the sodium formate mass spectrum can be found in the masslynx folder, opened with notepad from **C:\MassLynx\Ref** (note these are the same values referenced when making a calibration).

2.6 The chromatograms in Fig Figure 7 & Figure 8 show examples of the spectra you should see for Myoglobin and Sodium formate.

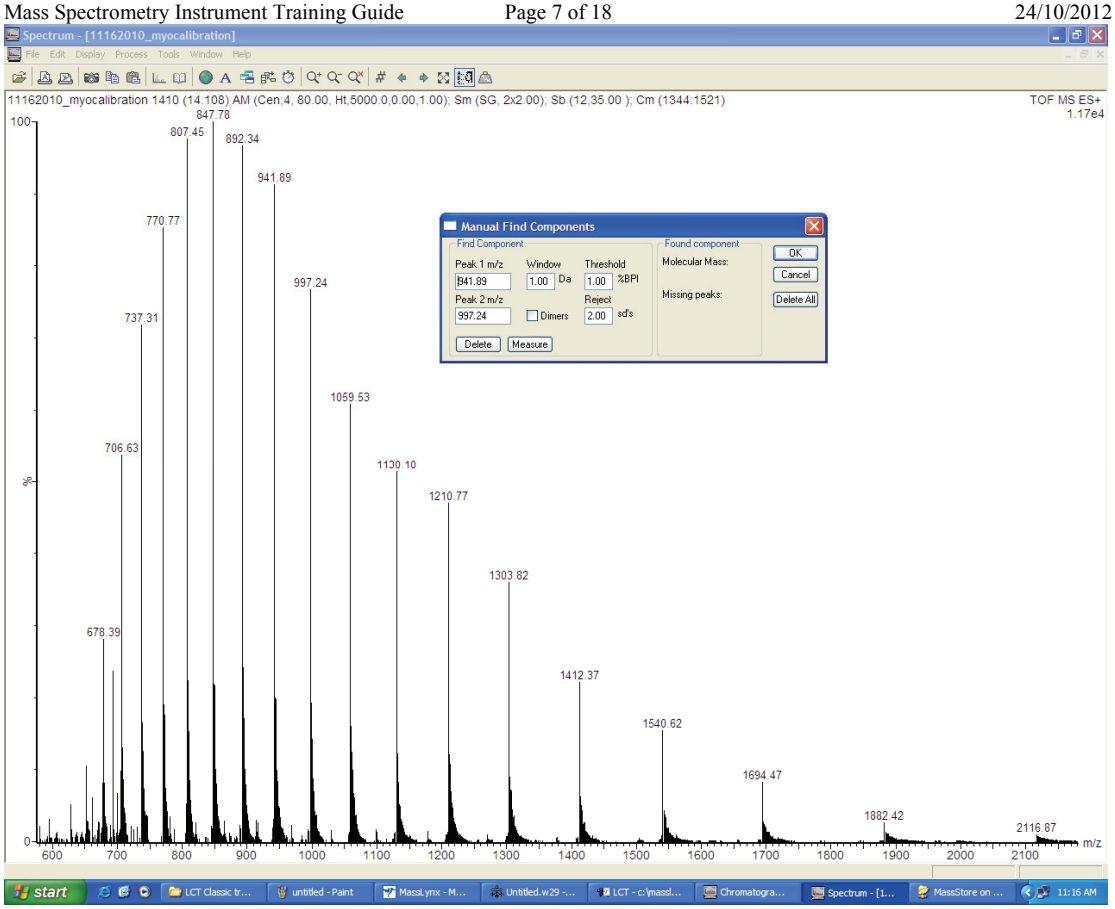



**Figure 7: Denatured Myoglobin spectra**

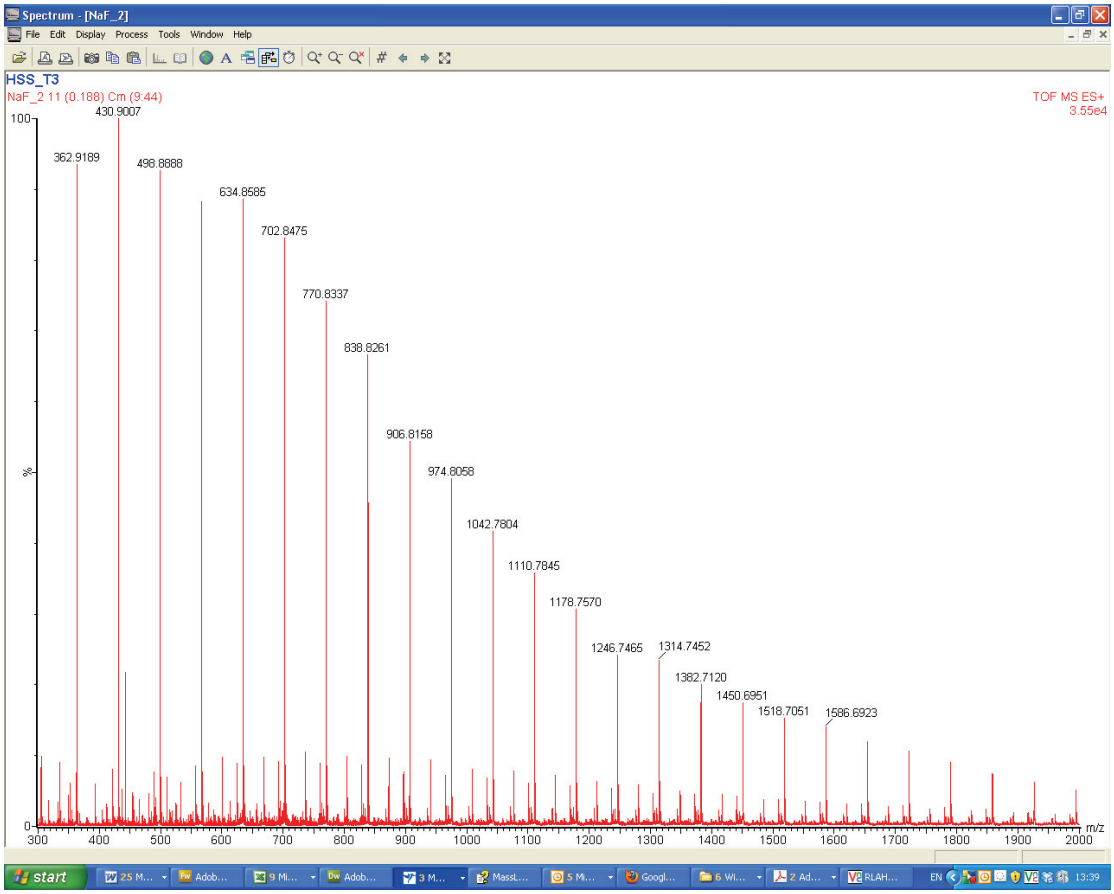



**Figure 8: Direct infusion of sodium formate in positive ion mode.**

2.7 To calibrate:

2.7.1 set cone voltage to 30V
2.7.2 Directly infuse sample using syringe pump at 5 or 10uL/min
2.7.3 Acquire data over the range 100 to 2000
2.7.4 Open Spectra (sum at least 10 scans) then process and centre the data: Process/subtract/smooth and centre (Figure 9).

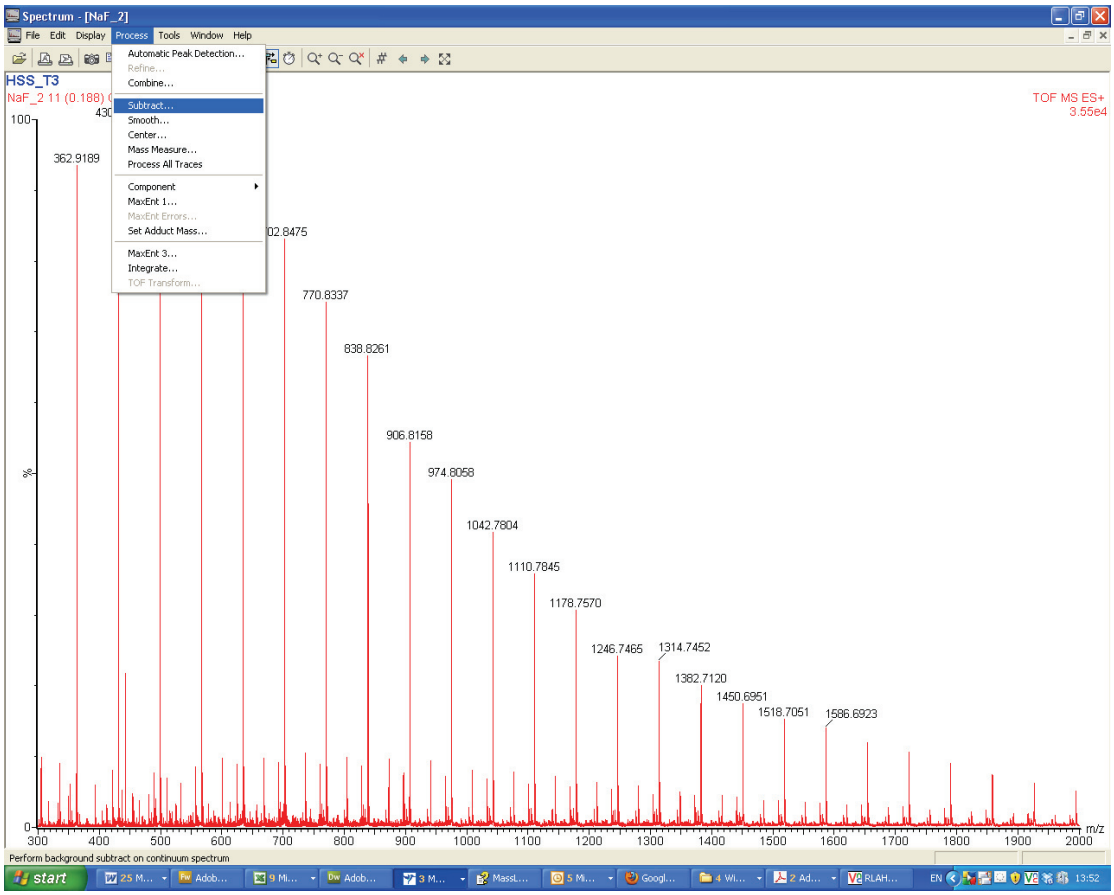



**Figure 9: Process mass spectra - subtract, smooth and centre.**

2.8 Spectrum: Go to Tools/Make Calibration/choose the NaF positive ion mode reference file and then manually match peaks (Figure 15).

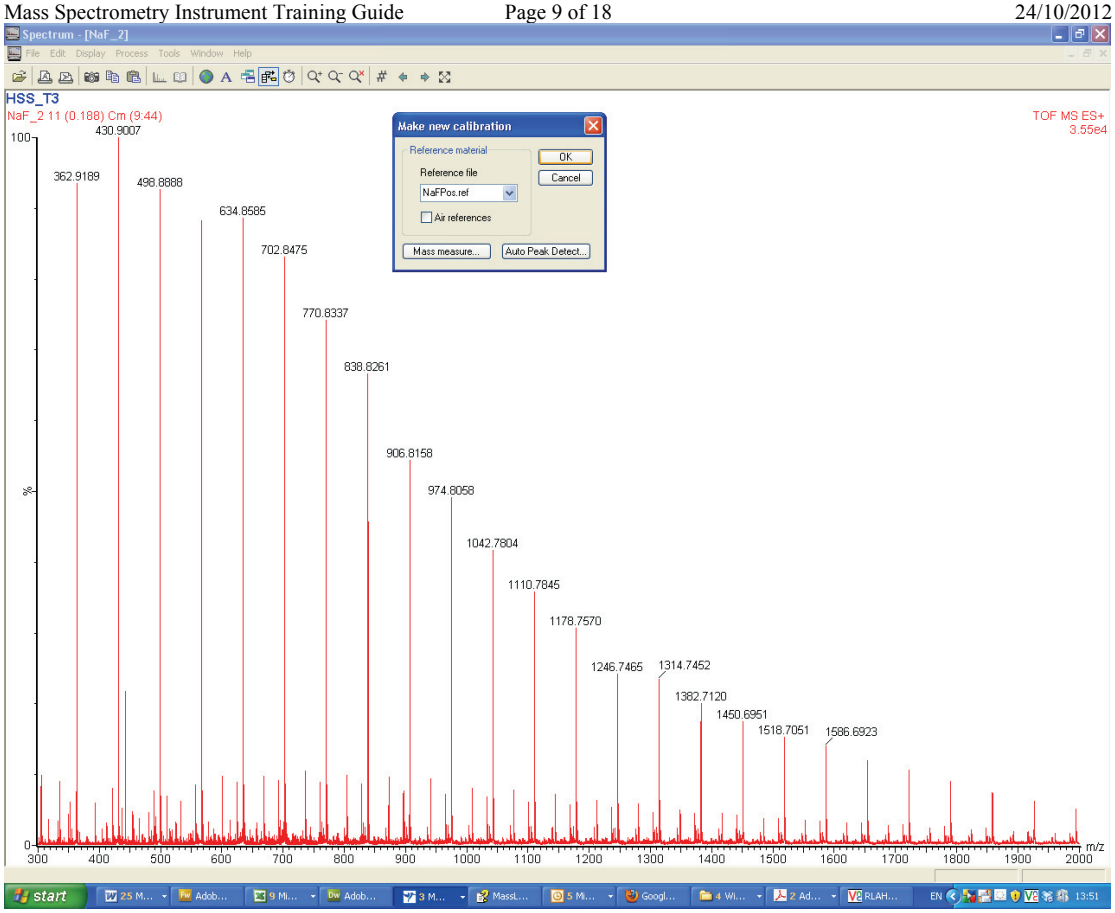



**Figure 10: Go to Tools/Make Calibration/choose the NaF reference file.**

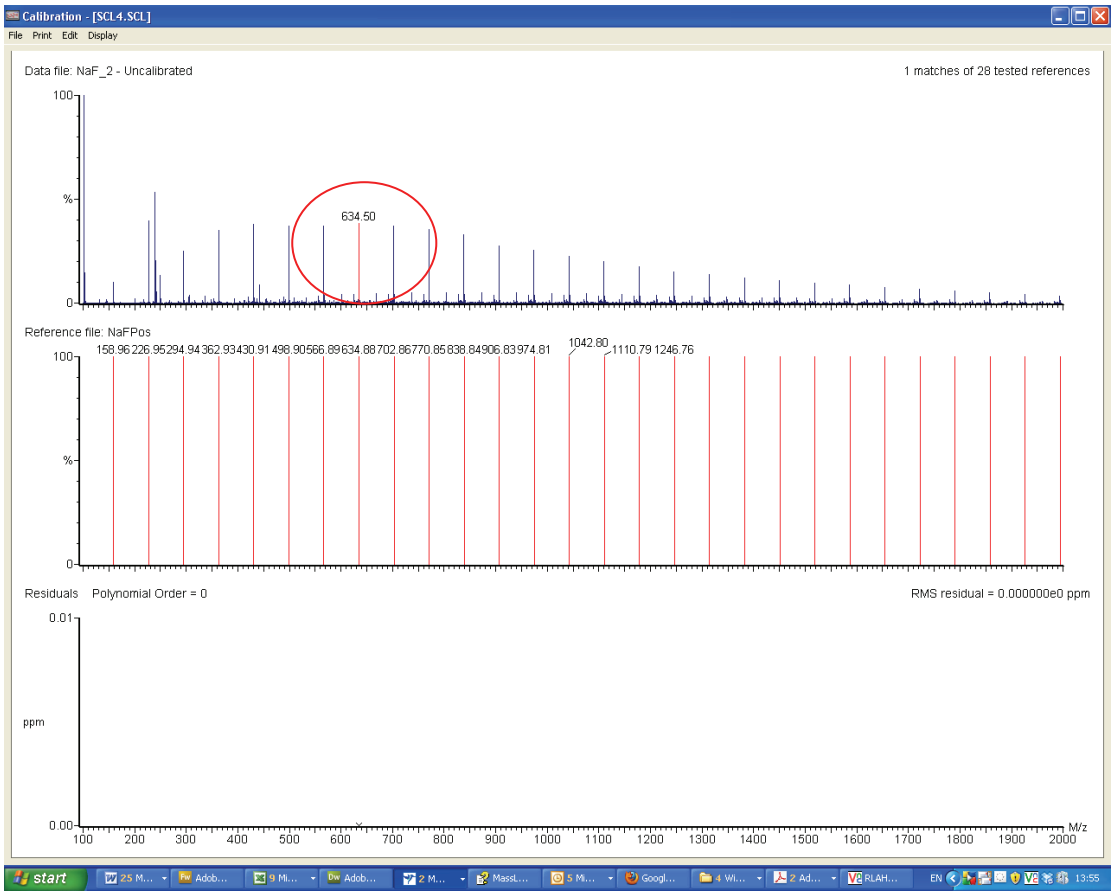



**Figure 11: Manually match calibration file peaks.**

2.9 Select File/save as/calibration: enter a filename, choose SAVE followed by OK.

2.10 Choosing 'OK' on the calibration page without first saving the calibration as a different file will automatically overwrite the current calibration.

## 3. Setting up an LC/MS sequence

3.1 Arrange basic plumbing with column inline after the UV cell (if using UV) and mass spec detection (Figure 12)

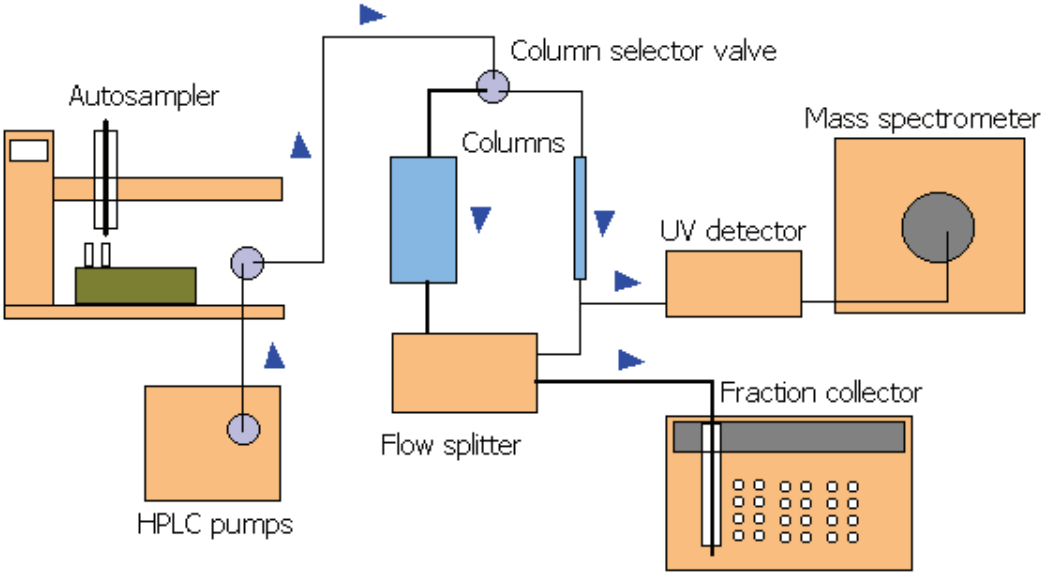



**Figure 12: an appropriate diagram for plumbing of HPLC**

3.2 The sample list is loaded on the main masslynx page. A new sample list can be opened or a new one started using: File/open or File/new respectively. Samples lists are kept in the 'Sampledb' subfolder in your main project folder. Sample lists can be copied or renamed.

3.3 The sample list has a line for each sample to be run and a large number of samples can be edited and run as a single batch.

3.4 Each line requires a sample name, a mass spec method, an inlet method (make sure all timings for the length of run match), a vial position for the sample and an injection volumne (note: the injection volume in the sample list overrides that given in the inlet method.

3.5 Editing Inlet method: There are 4 important tabs (circled in red in Figure 13) The first is the 'display status' this provides information about the flow rate, which pumps are running, their pressures and any faults with the inlet. Note the 'ready' and 'OK' light should both be green during normal operation. A red light in either signifies a fault as for the 'OK' light in Figure 13.

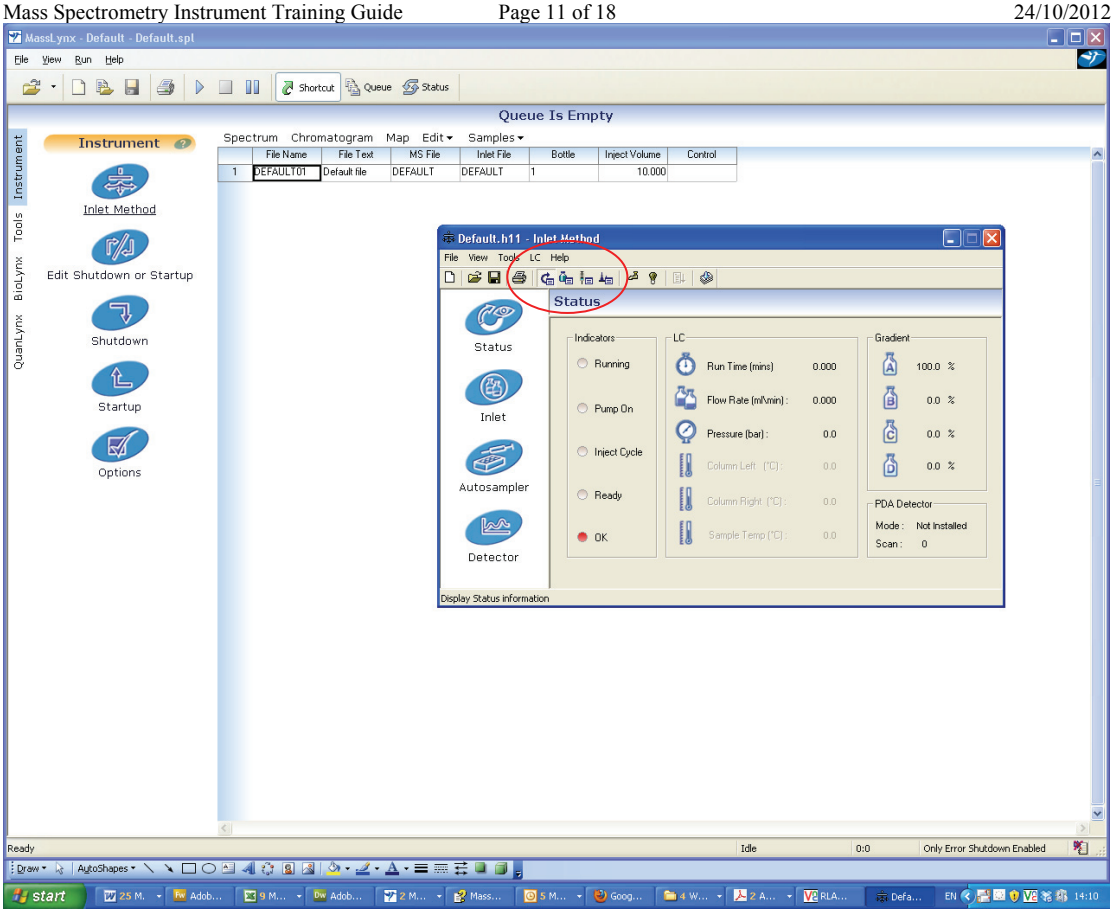



**Figure 13: There are 4 important tabs circled in red.**

3.6 Editing MS method: Right click and select edit on the 'MS File' group (Figure 14). This will open the MS method which can then be modified (alternatively click on MS method in the 'Instrument' section of the Main Masslynx page. Set the mass range for data collection and length of time mass spec data should be collected for. Note this does not have to be the same length as the LC method run time.

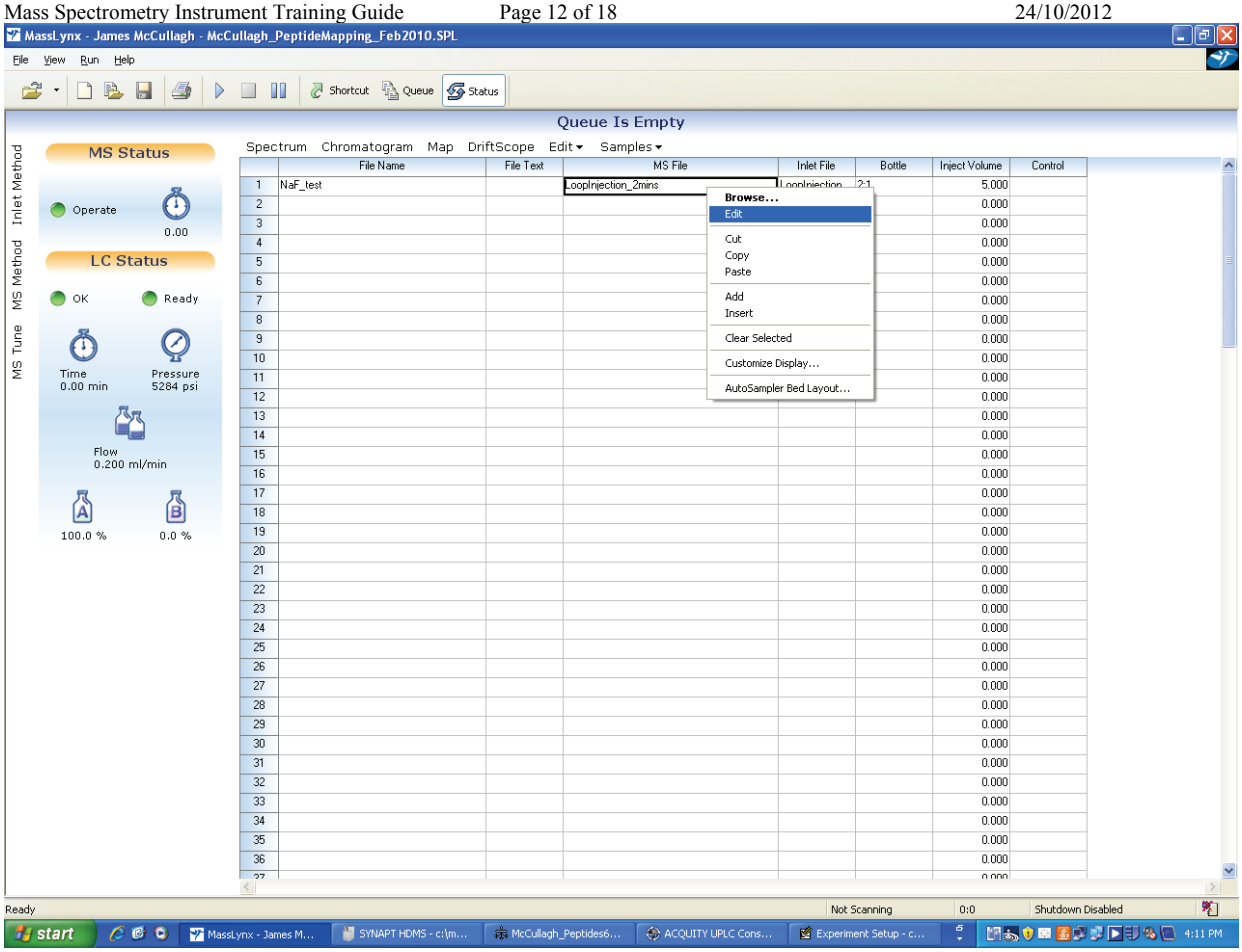



**Figure 14: Editing MS method from sample list.**

3.7 Editing and saving sample lists: The sample list can be saved and opened using: File/open.

3.8 labelling bottle/vial number in sample list: Make sure the sample list contains an injection volume and a vial position in the autosampler.

3.9 Start LC/MS run: Click on start on the MassLynx main page (make sure Files names are not repeated in the sample list and spaces/dissallowed characters are not present – avoid dots or commas, use underscore or dashes instead).

## 4. Using UV and MS detectors in sequence

4.1 To add the UV detector open the inlet file in the sequence (right click and open)

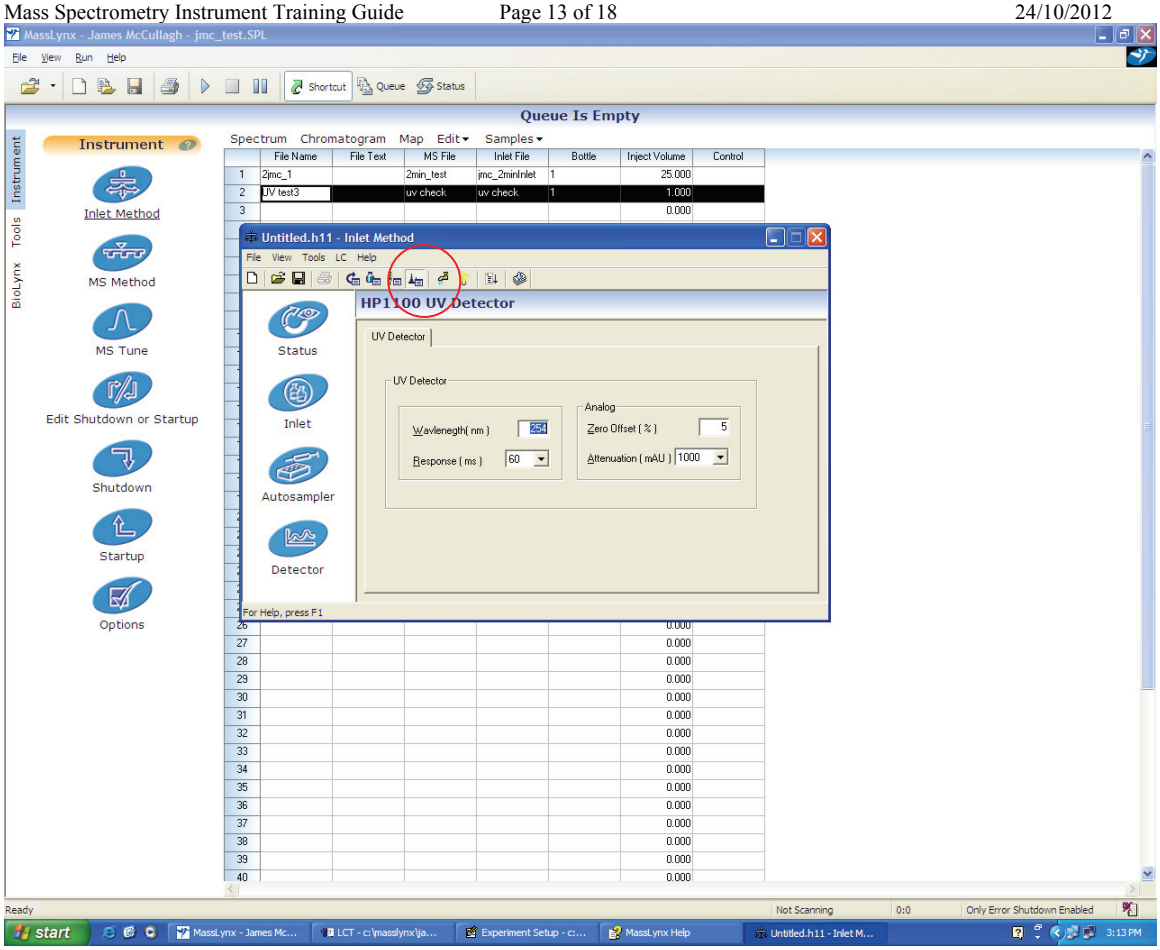



**Figure 15: Editing UV detector parameters**

4.2 Select the 'edit detector parameters' tab, see Figure 15.

4.3 Choose the wavelength required and save the inlet method.

4.4 To view the UV trace open the chromatogram window, select 'Display' from the menu bar and then Analogue… (Figure 16)

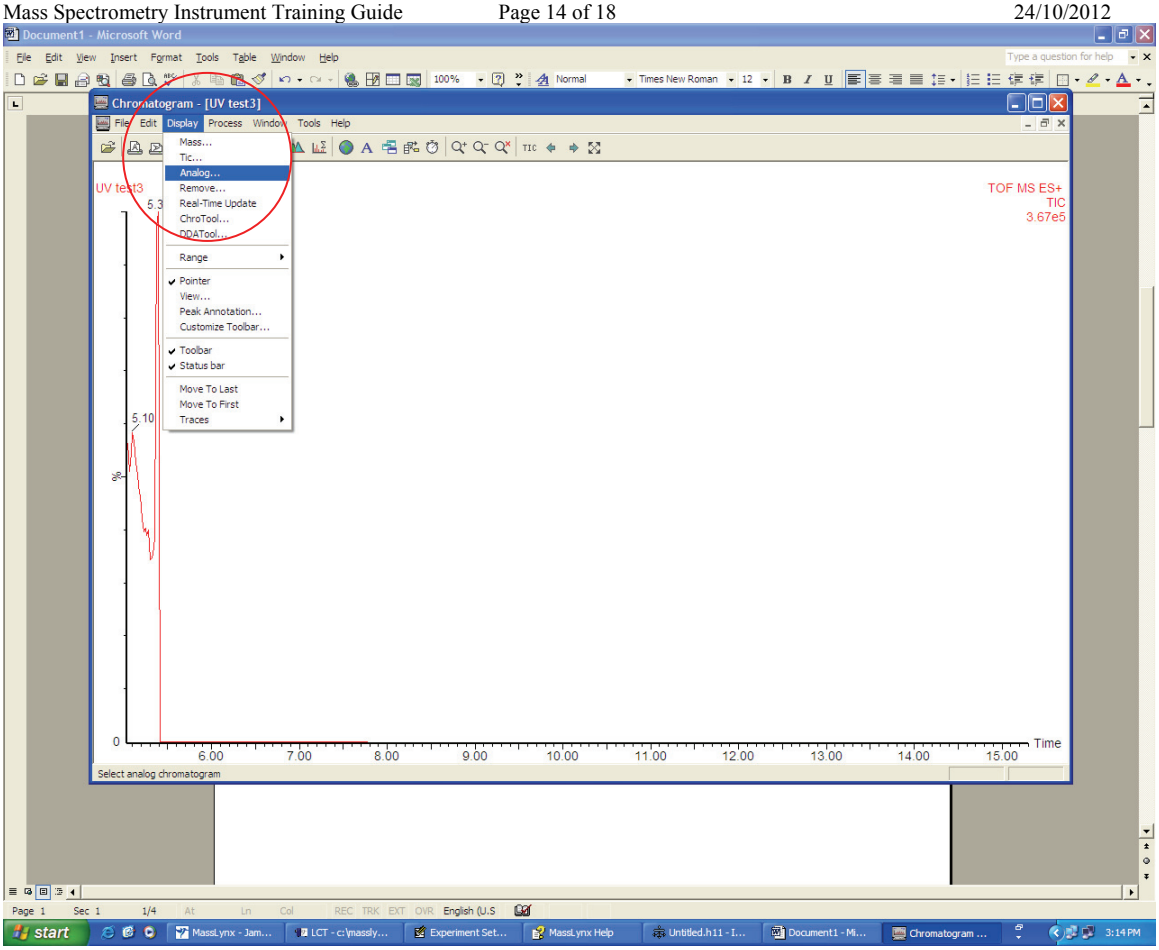



**Figure 16: Viewing UV trace through analogue channels**

4.5 Choose analogue channel 1 to display the channel which collected the UV data (Figure 17)

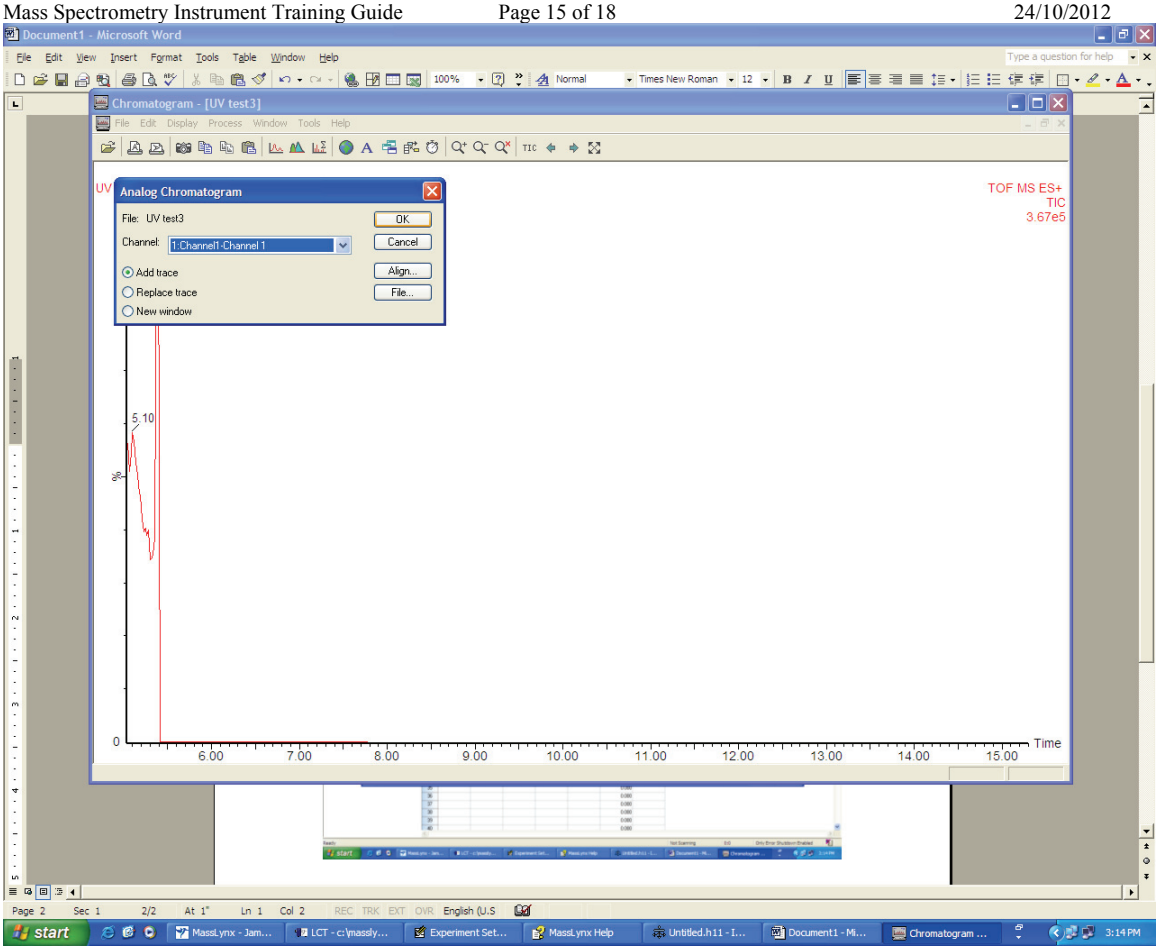



**Figure 17: Choose channel 1 to view UV trace.**

4.6 UV trace for the wavelength selected in the inlet method is added to or replaces the TIC trace in the chromatogram window (Figure 18).

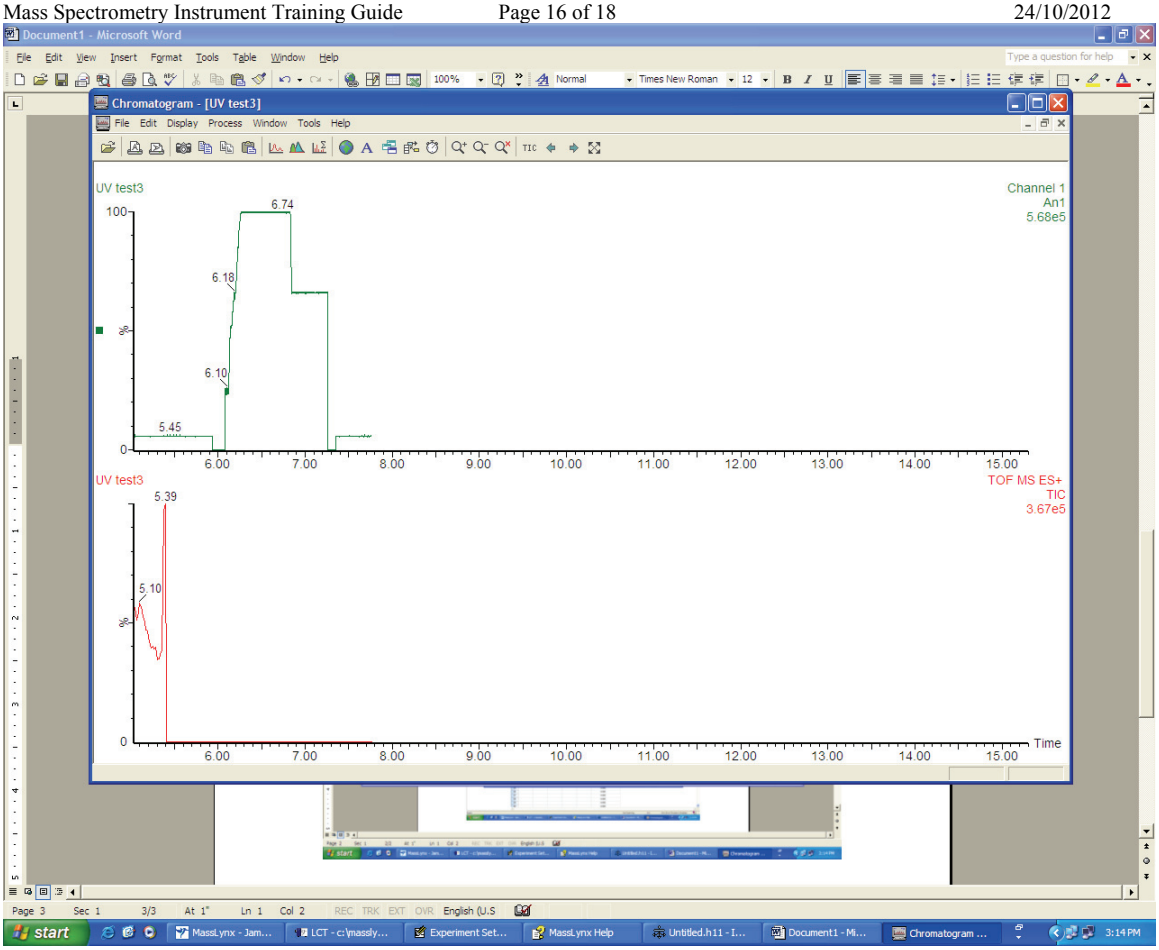



**Figure 18: UV trace for the wavelength selected in the inlet method is added to or replaces the TIC trace in the chromatogram window.**

## 5. Cleaning the sampling cone

5.1 The sample cone should be cleaned regularly whenever a discolouration can be seen around the orifice (this may be every 24 hours for continuous use or more frequently if protein samples or buffers are run at higher concentrations.

5.2 Before removing cone make sure isolation valve is closed.

5.3 The cone should be removed and each metal part sonicated for 20mmins in 50:50 MeOH with acetonitrile and 0.1% formic acid. There is a sonic bath in the basmement mass spec fume hood. Blow all parts dry with air before re-assembly.

5.4 After re-assembly **make sure isolation valve is open.**

## Removing and Cleaning the Sample Cone

*Caution:* The sample cone is a delicate and expensive component and should be handled with extreme care.

It is not necessary to vent the instrument to remove the sample cone. The source block incorporates an isolation valve for this purpose. To remove the sample cone proceed as follows:

Follow the procedure in the previous section, to gain access to the sample cone.

Using a suitable flat blade screwdriver rotate the valve by 90° into its fully anticlockwise position.

*A small improvement in the analyser vacuum may be observed as a result of this operation.*

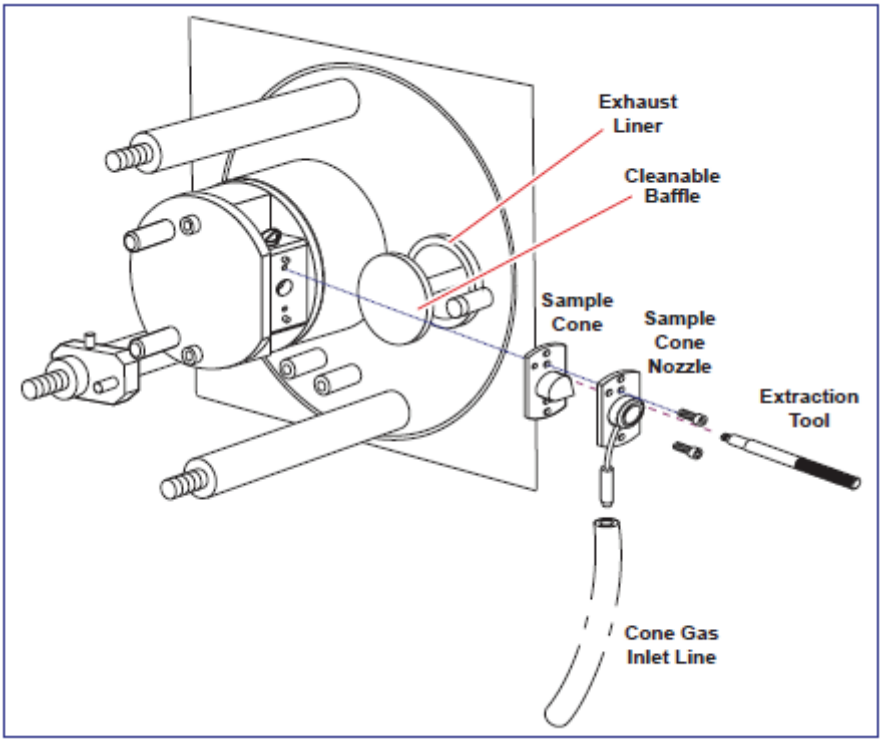
*The isolation valve is in the closed position when the slot is perpendicular to the direction of flow.*

Disconnect the cone gas inlet line (if fitted).

Take the sample cone extraction tool supplied in the source spares kit and screw it to the flange of the sample cone.

Remove the two sample cone retaining screws using a 1.5mm Allen key and withdraw the sample cone and cone gas nozzle (if fitted) from the ion block.

Remove the extraction tool, and separate the sample cone from the cone gas nozzle. Place both components in an ultrasonic bath containing 50:50 acetonitrile:water or 50:50 methanol:water.



Dry the cone and nozzle using nitrogen.

*To minimise down time fit a spare sample cone, obtainable from Micromass, at this stage.*

If material has built up on the exhaust liner and cleanable baffle:

Remove the cleanable baffle and the exhaust liner.

*Caution:* Do not attempt to remove the baffle without first removing the sample cone.

Clean these components, or obtain replacements.

Fit the cleaned (or the replacement) exhaust liner and cleanable baffle to the ion block.

Refitting the sample cone is a reversal of the removal procedure.

## 6. Contacting MS staff

For instrument related questions or if you have a problem with using the LCT contact one of the mass spectrometry research technicians; James Wickens, Colin Sparrow or Lingzhi Gong in the basement mass spec lab on 75710.

Alternatively or for any other enquiries please contact: Dr James McCullagh (Head of the Mass Spec Lab) on 75657.